Please substitute the paragraph at page 12, lines 12-15 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

C7

Genes encoding phosphatase include those derived from *Morganella morganii* (Japanese Published Unexamined Patent Application No. 37785/97, Japanese Published Unexamined Patent Application No. 201481/98), etc.

Please substitute the paragraph starting at page 12, line 30 and ending at page 13, line 4 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

C8

In the case of the gene encoding inosine-guanosine kinase, primers are synthesized based on the sequences at both ends of the sequence of an inosine-guanosine kinase structural gene, and the inosine-guanosine kinase structural gene can be obtained by the PCR method using the prepared primers and the *Escherichia coli* chromosomal DNA or the *Exiguobacterium acetylicum* chromosomal DNA. Similarly, by the use of the PCR method, a phosphatase structural gene can be obtained from the *Morganella morganii* chromosomal DNA and an adenylate kinase structural gene can be obtained from the *Saccharomyces cerevisiae* chromosomal DNA.

REMARKS

The claims have been amended in order to recite the present invention with the specificity required by statute. Additionally, the specification has been amended to delete "acid" inserted in the July 10, 2001 Amendment. The subject matter of the

amendment maybe found in the specification as filed, inter alia, at page 12, line 12-15 referring to Morganella morganii.[1/] Accordingly, no new matter has been added.

I. The specification is objected to and the claims rejected under 35 U.S.C. §132.

In support of the rejection, the Examiner contends there is no literal or inherent support for the term "acid phosphatase" as noted in paragraph 18 of the Office Action. Although this rejection is respectfully traversed, solely in order to reduce the issues, the term has been deleted. Accordingly, this rejection is overcome.

II. Claims 1 and 5-8 (and new claim 20) remain rejected under 35 U.S.C. §112, second paragraph, as indefinite.

Specifically, the Examiner states that most biosynthetic pathways are integrated with other biosynthetic pathways, the purine nucleotide synthetic pathway is not identified, and it is unclear, for instance, if sugar biosynthesis is included as precursors. In

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1/ EP0832970A1 corresponds to Japanese Published Unexamined Patent Application No. 37785/97 noted thereat which teaches on page 3, lines 13 to 32 that Morganella morganii derived phosphatase can be used to produce nucleoside-5' -phosphate ester.

> "It is noted that acid phosphatase (EC 3.1.3.2) is originally an enzyme which catalyzes a reaction to hydrolyze phosphate ester under an acidic condition, and it has a nucleotidase activity to degrade nucleoside-5' -phosphate ester produced by the transphosphorylation reaction. Even such an acid phosphatase can be used in the method for producing nucleoside-5' -phosphate ester of the present invention."

response, this rejection is overcome by the above amendment specifying the most preferred embodiments of the precursors intended herein. Accordingly, this rejection is mooted.

III. Claims 3 and 4 are newly rejected as indefinite under 35USC§112, second paragraph.

The Examiner states that previously she interpreted the claims to read on a single enzyme, and now interprets that two enzymes are required, thus now it is not clear how a phosphatase can catalyze production of a nucleotide. This rejection too is mooted by the above amendment.

IV. Claims 1, 2, 5 and 7 remain rejected under 35 U.S.C. §102 as anticipated by Fujio.

In support of the rejection, The Examiner states as follows:

> "Fujio et al. teach a method of production of GMP using E. coli transformed with a gene for GMP synthetase (XMP aminase) under the control of the temperature-sensitive PL promoter (see page 842, right column). E. coli inherently contain XMP, a precursor; and Fujio et al. teach their method as industrial production of GMP, thus inherently containing a recovery step."

Applicants have taken note of the Examiner's comment and amended Claim 1 accordingly. As a result, the Examiner's statement that E. coli inherently contain XMP is essentially irrelevant insofar as the language of the pending claims. That is, while E. coli has a pathway for purine nucleotide synthesis and inherently contains some XMP as a metabolite, the XMP is utilized and consumed in the metabolic pathway and is not secreted through the cell membrane to accumulate in the culture, as required by the present amended claims.

As noted, claim 1 is amended to recite culturing a transformant to accumulate in culture a purine nucleotide precursor, specifically XMP, guanosine, inosine or adenosine. This feature of the present invention is simply not taught by Fujio because (even if E. coli contains XMP), the XMP is used metabolically and is not secreted, let alone accumulated in culture.

V. Claims 1 and 3-6 remain rejected as anticipated by Usuda (EP 0816491).

The Examiner states that Usuda teaches in Example 1 a method of making IMP and GMP by transforming C. ammoniagenes with a gene encoding inosine-guanosine kinase. Usuda et al. relates to a process for producing 5' -inosinic acid or 5' -guanylic acid from inosine or guanosine or a precursor thereof using adenosine triphosphate(ATP)-producing microorganisms containing a DNA encoding inosine-guanosine kinase. In Usuda, the enzyme inosine-guanosine kinase is constitutively expressed, e.g., the enzyme is produced constantly in a fixed amount regardless of growth conditions. In such case, the reactions of IMP → inosine and inosine → IMP simultaneously occur and IMP is not accumulated in culture because of the IMP's poor membrane permeability. This deficiency is remedied in Usuda by the addition of inosine (the precursor of IMP) from the outside in a purified preparation form.

However, the present invention differs by accumulating in culture a precursor of the purine nucleotide used for the following step of converting the accumulated precursor into the purine nucleotide by an enzyme upon induction. Usuda does not teach this feature.[2/]

VI. Claims 7, 8 and 20 are rejected under 35 U.S.C. §103 as being obvious over Usuda in view of Katsumata (U.S. 5,439,822). Additionally, claims 8 and 20 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Fujio et al. or Usuda et al., either in view of Katsumata et al.

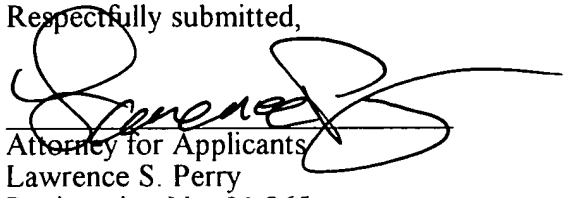
The deficiencies of Usada and Fujio regarding the amended claims are discussed above. None of these deficiencies is overcome by Katsumata, which is cited only as showing use of an ICL promoter for use in acetate-induction expression systems in Coryneform and E. coli.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

2/ Moreover, Usuda (and the other prior art) teach away from accumulating a purine nucleotide. For instance, guanosine is biosynthesized in a microorganism by the pathway of IMP → XMP → GMP → guanosine. IMP dehydrogenase (which catalyzes the reaction of IMP → XMP) is strongly inhibited by the feedback inhibition of GMP. Therefore, if produced guanosine is converted to GMP with inosine-guanosine kinase in the microorganism, the reaction of IMP → XMP is inhibited by the converted GMP and as a result the synthesis of XMP is stopped.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should be directed to our below listed address.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

1. (Three Times Amended) A process for producing a purine nucleotide which comprises:

culturing in a medium a recombinant microorganism obtained by transforming a host cell having the ability to produce a precursor of the purine nucleotide selected from the group consisting of XMP, guanosine, inosine and adenosine with DNA which can express an enzyme capable of synthesizing the purine nucleotide from said precursor upon induction, to accumulate said precursor of the purine nucleotide in the culture;

inducing the expression of the enzyme capable of synthesizing the purine nucleotide from said precursor to form the purine nucleotide from the accumulated precursor in said culture, and

recovering the formed purine nucleotide therefrom.

3. (Twice Amended) The process according to claim 1, wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or [acid] phosphatase derived from Morganella morganii, and the purine nucleotide is 5'-guanylic acid.

4. (Twice Amended) The process according to Claim 1, wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or [acid] phosphatase derived from Morganella morganii, and the purine nucleotide is 5'-inosinic acid.

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

The paragraph starting at page 3, line 25 and ending at page 4, line 4 has been amended as follows:

As for the phosphorylation, methods using phosphotransferase, kinase and phosphatase are known. In particular, the reaction utilizing kinase or phosphatase has been studied as an efficient method. For example, there have been developed a process for producing a 5'-nucleotide by the use of an *Escherichia coli* strain carrying a gene encoding inosine-guanosine kinase of *Escherichia coli* (W091/08286), a process for producing a 5'-nucleotide by the use of a *Corynebacterium ammoniagenes* strain carrying a gene encoding inosine-guanosine kinase of *Exiguobacterium acetylicum* (W096/30501), and a process for producing a 5'-nucleotide by the use of an *Escherichia coli* strain carrying a gene prepared imparting a random mutation to the [acid] phosphatase gene of *Morganella morganii* (Japanese Published Unexamined Patent Application No. 37785/97, Japanese Published Unexamined Patent Application No. 201481/98).

The paragraph at page 7, lines 11 through 20 have been amended as follows:

(3) The process according to the above (1), wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or [acid] phosphatase, and the purine nucleotide is 5'-guanylic acid.

(4) The process according to the above (1), wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide

from said precursor is inosine-guanosine kinase or [acid] phosphatase, and the purine nucleotide is 5'-inosinic acid.

The paragraph at page 8, lines 21 through 30 have been amended as follows:

(15) The microorganism according to the above (13), wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or [acid] phosphatase, and the purine nucleotide is 5'-guanylic acid.

(16) The microorganism according to the above (13), wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or [acid] phosphatase, and the purine nucleotide is 5'-inosinic acid.

The paragraph starting at page 11, line 31 and ending at page 12, line 1 has been amended as follows:

As the enzyme capable of synthesizing a purine nucleotide from its precursor to be used in the present invention, any enzyme capable of synthesizing a purine nucleotide from its precursor can be used, and suitable examples include XMP aminase, inosine-guanosine kinase, [acid] phosphatase and adenylate kinase.

The paragraph at page 12, lines 12-15 have been amended as follows:

Genes encoding [acid] phosphatase include those derived from *Morganella morganii* (Japanese Published Unexamined Patent Application No. 37785/97, Japanese Published Unexamined Patent Application No. 201481/98), etc.

The paragraph starting at page 12, line 30 and ending at page 13, line 4 has been amended as follows:

In the case of the gene encoding inosine-guanosine kinase, primers are synthesized based on the sequences at both ends of the sequence of an inosine-guanosine kinase structural gene, and the inosine-guanosine kinase structural gene can be obtained by the PCR method using the prepared primers and the *Escherichia coli* chromosomal DNA or the *Exiguobacterium acetylicum* chromosomal DNA. Similarly, by the use of the PCR method, a [acid] phosphatase structural gene can be obtained from the *Morganella morganii* chromosomal DNA and an adenylate kinase structural gene can be obtained from the *Saccharomyces cerevisiae* chromosomal DNA.